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14. ABSTRACT

Scope: To investigate the role of early life processes, endocrine mediators and number of susceptible cells on adult life breast cancer risk. Method: Five interlinked component projects covering the spectrum from endometrial to adult life. Progress report: Component projects 1 to 4 were officially launched July 2005. Tasks and subtasks to be performed were described in the submitted Statement of Work (SOW). Subtasks 1a, 1b, 2a, 2b, 3a, 3b, 3c, 4a and 4b are currently ongoing. Subtasks 6a and 6b under Task 6 are being implemented. Major findings: Data collection is still on-going. Analyses are pending and no findings can be reported yet.

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Breast cancer, early life, mammary gland specific stem cells, hormones

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INTRODUCTION

The aim of the project is to investigate the role of early life processes, endocrine mediators and number of susceptible cells on adult life breast cancer risk. Based on the hypothesis that breast cancer risk is a function of number of mammary gland cells at risk of transformation and that this number is largely modulated by perinatal events and conditions, five component projects have been initiated. The first three focus on perinatal characteristics, including immediate postnatal growth, in relation to mammary gland mass and breast cancer risk, whereas the last two explore the relation of pregnancy hormones with breast cancer risk and with cellular populations that are likely to have mammary stem cell potential. The five projects are interlinked and they address the hypothesis that growth and mammotropic hormones in perinatal life affect the number of susceptible mammary gland cells. This number is likely to be reflected in birth size and rate of postnatal growth that, in turn, represent intermediate steps and correlates of mammary gland mass and breast cancer risk in adult life. The progress on each component project (CP) will be reported separately to facilitate the reader.

BODY

CP1 "Association of growth during the first postnatal week with breast cancer risk in adult life"

CP1 PI: Prof. Anders Ekbom, Unit of Clinical Epidemiology, Dept. of Medicine, Karolinska Institutet/Karolinska University Hospital, SE-171 76 Stockholm, Sweden.

Timetable of research accomplishments of CP1 as outlined in the Statement of Work.

Task 1 *To investigate the association of growth during the first postnatal week with breast cancer risk in adult life:*

a. Retrieval of available birth records from 1,068 women with incident breast cancer and 2,727 control women. (Months 1-24)
b. Extraction of data on growth of newborns during the first postnatal week, as well as information on covariates to be used in the analysis. (Months 25-30)
c. Linkage of data on postanatal growth and perinatal covariates to cancer and mortality registries. (Months 31-36)
d. Data analyses. (Months 37-48)
e. Manuscript preparation and submission. (Months 49-60)

CP1 progress report

Component project 1 was officially launched July 1, 2005. The retrieval of birth records (task 1a) and extraction of data on growth of newborns (task 1b) started in late fall 2005 and are on schedule. In the retrieval process, the extraction of data on growth is done in parallel in newborns through the first post natal week. We have so far encountered no problems to identify and retrieving information as given in the research plan.

Task 1c, d and e has not been initiated according to the time plan.

CP1 key research accomplishments

- The retrieval of birth records from 1,068 women with incident breast cancer and 2,727 control women has started.
- The extraction of data on growth of newborns during the first postnatal week has started.

CP1 reportable outcomes

No outcomes can be reported at this stage.

CP1 Conclusion

No major problems have been encountered and none are foreseen for the continuation of the implementation of the component project.

CP2: "Relation of perinatal characteristics and postnatal growth velocity with mammographic patterns in adult life"

CP2 PI: Prof. Per Hall, Dept. of Medical Epidemiology and Biostatistics, Karolinska Institutet, P.O. Box 281, SE-171 77 Stockholm, Sweden

Timetable of research accomplishments of CP2 as outlined in the Statement of Work.

Task 2 To investigate the relation of perinatal characteristics and postnatal growth velocity with mammographic patterns in adult life:

a. Retrieval of available birth records from 3,345 women with invasive breast cancer and 3,454 controls (these women are not the same with those to be studied in the context of task 1). (Months 1-48)
b. Retrieval of the available sequential mammographies of the women with breast cancer and the control women. (Months 1-48)
c. Linkage of mammographic data to perinatal characteristics and postnatal growth velocity. (Months 30-48)
d. Evaluation of mammographies through a computer-assisted grey-scale thresholding methods technique. (Months 25-36)
e. Data analyses. (Months 37-48)
f. Manuscript preparation and submission. (Months 49-60)

CP2 progress report

Component project 2 was officially launched July 1, 2005.

The retrieval of birth records (task 2a) has been initiated. By the end of 2005, a person was hired for this task and she has so far spent most of her time identifying where the individuals were born. Through the taxation authorities, it is in Sweden possible to identify where a person was born and where a person has been living during their life-time.

The retrieval of available mammograms (task 2b) has started. Data abstractors were engaged in August. In the initial phase, we have focused on the Stockholm area (inhabited by 2 out of 9.1 million Swedes). For the Stockholm area, we have access to information on where all women participated in screening mammograms the last 20 years. We have therefore matched our data set to the screening register and started to collect mammograms. We also have to manually search the 14 archives in Stockholm since we do not have information on where clinical mammograms were performed for the control women. In a previous study, where the only aim was to collect information on where, when and why a mammogram was taken, this information was collected for the case subjects.

We have established collaboration with the Department of Radiology, Karolinska University Hospital. Drs. Gunilla Svane and Edward Azavedo will participate in the evaluation of the mammograms but also have the overall responsibility for quality assurance of the study. As an example, one problem is if copies of the mammograms will be equally good as originals when it comes to estimating density of the breast. The experience is that it will but we intend to perform a feasibility study where originals and copies from the same mammograms are estimated and validated.

After a mammogram has been copied, it has to be scanned in order for the density measurement to be performed. We will in the initial phase of the study use the scanner at the Department of Radiology. Given the large number of mammograms, it is likely that we will buy our own scanner.

Task 2c-f. These tasks have not yet been initiated.

CP2 key research accomplishments

- Addresses from the taxation authorities, enabling us to retrieve birth records, have been received.
- The time and place where patients had their mammographies taken (screening only) have been identified and copying of mammographies has started.

CP2 reportable outcomes

At this stage, there are no reportable outcomes.

CP2 Conclusion

As outlined under “Research accomplishments”, we are well on the way with the goals for task 2. It has taken us somewhat longer than anticipated to get information on birth place and mammography use. However, we are now up and running and have the necessary qualifications and manpower. No major problems have been encountered and none are foreseen for the continuation of the implementation of the component project.

CP3: “Interaction of perinatal characteristics with genes that are likely related to breast cancer risk”

CP3 PI: Prof. Per Hall, Dept. of Medical Epidemiology and Biostatistics, Karolinska Institutet, P.O. Box 281, SE-171 77 Stockholm, Sweden

Timetable of research accomplishments of CP3 as outlined in the Statement of Work.

Task 3 To investigate the possible interaction of perinatal characteristics with genes that are likely related to breast cancer risk:

a. Identification and selection of genes likely to be related to breast cancer risk, e.g. ESR1, AIB1, and the IGF family (Months 1-12)
b. Selection of "tagging" single nucleotiod polymorphisms (tSNPs). The choice of tSNBs aims at avoiding redundant genotyping. A good marker coverage is expected to be achieved by using approximately one SNP per 3 Kb. (Months 1-12).
c. Genotyping of the approximately 8 genes selected for the study (Months 13-36).
d. Data analyses. (Months 36-48)
e. Manuscript preparation and submission. (Months 49-60)

CP3 progress report

A high percentage of dense parenchyma on mammographic images, which appears to confer a 4- to 6-fold increased risk for breast cancer, has a genetic component, based on the significant correlations with breast density observed between sisters and the 2-fold higher correlation between monozygotic compared to dizygotic twins. A role of steroid hormones in mammographic density is supported by observations of an increase in density after HRT and a decrease after suppression of ovarian function through a gonadotropin-releasing hormone agonist or after tamoxifen treatment. Genetically determined differences in biosynthesis and metabolic pathways of estrogens may affect breast cancer risk as reflected in mammographic density. We focused on the association of polymorphisms in ESR1, AIB1 and the IGF family.

Component project 3 was officially launched July 1, 2005.

DNA has been isolated for approximately 50% of the cases and controls described in task 2a. The DNA has been shipped to the Genome Institute of Singapore where genotyping will be performed. The genes that we will focus on are ESR1, ATM, IGF-1, CHEK2, HER-2. The CHEK2 gene has already been analyzed in a straight-forward association study. When we have

the mammography information, polymorfisms in these genes will be related to mammographic pattern.

The selection of tSNPs (task 3b) has been identified for the CHEK2 gene and is currently ongoing for the other genes mentioned under task 3a.

Genotyping (task 3c) has already been finished for the CHEK2 gene and will start shortly for the other genes mentioned under task 3a.

Task 3d-e. These tasks have not yet been initiated.

CP3 key research accomplishments

- SNP selection, genotyping, and identification of haplotype tagging SNPs has started.
- Genotyping for the CHEK2 gene has been completed and genotyping for the other genes mentioned under task 3a will start shortly.

CP3 reportable outcomes

At this stage, there are no reportable outcomes.

CP3 Conclusion

As outlined under "Research accomplishments", we are well on the way with the goals for task 3. We are now up and running and have the necessary qualifications and manpower. No major problems have been encountered and none are foreseen for the continuation of the implementation of the component project.

CP4: "Pregnancy hormones and perinatal breast cancer risk factors in Boston, USA and Shanghai, China"

CP4 co-PIs: Prof. Dimitrios Trichopoulos and Dr. Pagona Lagiou, Department of Epidemiology, Harvard School of Public Health, 677 Huntington Avenue, Boston, MA 02115,

Timetable of research accomplishments of CP4 as outlined in the Statement of Work.

Task 4 To study maternal and cord blood levels of components of the IGF system and adiponectin among Caucasian women in North America and Chinese women in Asia in conjunction to maternal anthropometry and birth size parameters:

a. Retrieval of the available stored cord blood and maternal serum samples from 304 pregnant Caucasian women in Boston, US and 335 pregnant Chinese women in Shanghai, China and transfer of these samples to the laboratory for hormone determination. (Months 1-6)

b. Conduct of laboratory assays for hormones (Months 7-24)
c. Linkage of maternal and newborn data to maternal and cord blood hormone levels (Months 24-30)
d. Data analyses (each of the measured hormones in maternal and cord blood to be studied in conjunction to maternal and newborn variables) (Months 31-48)
e. Manuscript preparation and submission. (Months 37-60)

CP4 progress report

Component project 4 was officially launched July 1, 2005. With respect to baseline data, the database is already in place. A total of 1,376 blinded maternal serum samples have been retrieved from among the stored samples. The labeled sample numbers on the aliquots were recorded first before the samples were submitted to the ILAT Steroid RIA Laboratory, Department of Physiology, UMass Medical School, for hormonal assays. As the samples are identified only with a serum sample number, not the study ID, they include some that might be ineligible, duplicates, or with information missing on relevant study variables. These will be verified at the data merging stage before statistical analyses. Stored cord blood samples are still in the process of being retrieved and recorded before the laboratory assays begin. The laboratory has thus far assayed testosterone and IGF-1 on 232 maternal samples, IGFBP-3 on 231 maternal samples and adiponectin on 153 maternal samples.

CP4 key research accomplishments

- Available stored maternal serum samples have been retrieved and transferred to the laboratory for hormone determination. This process is currently being finalized for cord blood samples.
- Determination of IGF-1, IGFBP-3, adiponectin and testosterone in maternal serum samples has started.

CP4 reportable outcomes

At this stage, there are no reportable outcomes.

CP4 Conclusion

No major problems have been encountered and none are foreseen for the continuation of the implementation of the component project.

CP5: "Pregnancy hormones and perinatal breast cancer risk factors in Boston, USA and Shanghai, China"

CP5 PI: Prof. Chung-Cheng Hsieh, University of Massachusetts Cancer Center, 55 Lake Avenue North, Worcester, MA 01655

Timetable of research accomplishments of CP5 as outlined in the Statement of Work.

Task 5 To investigate whether markers of mammary stem cells are associated with perinatal characteristics that are linked to breast cancer in later life:

a. Finalization of questionnaire for obtaining maternal and gestation characteristics. (Months 1-3)
b. Training of the study personnel on study procedures. (Months 1-6)
c. Subject recruitment and sample collection from a total of 250 pregnant women. (Months 7-42)
d. Conduct of laboratory assays for markers of stem cells (Months 7-45)
e. Conduct of laboratory assays for hormones (Months 10-48)
f. Data analyses. (Months 45-54)
g. Manuscript preparation and submission. (Months 49-60)

CP5 progress report

At the time of the compilation of this report the clearance of component project 5 by the US Army Human Subjects Research Review Board (HSRRB) was being finalized. Due to this, component project 5 had not yet been launched.

CP5 key research accomplishments

- IRB approvals have been obtained following modifications in protocols and questionnaire used for assessing maternal and gestational characteristics (CP5).

CP5 reportable outcomes

At this stage, there are no reportable outcomes.

CP5 Conclusion

Launching of component project 5 has been delayed, but no major problems are foreseen for its implementation.

Task 6: "Monitoring, coordination and fine-tuning of the five component projects"

Monitoring and coordination of the five component projects has presented no problems. The key investigators have a long history of successful scientific collaboration, which continues in the context of the current project.

On November 8-9, 2005, a plenary meeting of all key investigators took place at the Department of Medical Epidemiology and Biostatistics, Karoliska Institutet, Sweden. Fine-tuning of the

component projects was discussed and decisions for the advancement of the research activities were made.

KEY RESEARCH ACCOMPLISHMENTS

- The retrieval of birth records from 1,068 women with incident breast cancer and 2,727 control women has started (CP1).
- The extraction of data on growth of newborns during the first postnatal week has started (CP1).
- Addresses from the taxation authorities, enabling us to retrieve birth records, have been received (CP2).
- The time and place where patients had their mammographies taken (screening only) have been identified and copying of mammographies has started (CP2).
- SNP selection, genotyping, and identification of haplotype tagging SNPs has started (CP3).
- Genotyping for the CHEK2 gene has been completed and genotyping for the other genes mentioned under task 3a will start shortly (CP3).
- Available stored maternal serum samples have been retrieved and transferred to the laboratory for hormone determination. This process is currently being finalized for cord blood samples (CP4).
- Determination of IGF-1, IGFBP-3, adiponectin and testosterone in maternal serum samples has started (CP4).
- IRB approvals have been obtained following modifications in protocols and questionnaire used for assessing maternal and gestational characteristics (CP5).

REPORTABLE OUTCOMES

At this stage, there are no reportable outcomes.

CONCLUSIONS

Launching of component project 5 has been delayed, but overall no major problems have been encountered and none are foreseen for the continuation of the implementation of the project.

REFERENCES

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APPENDICES

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